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(71)Applicant : CREAVIS G FUER TECHNOL & INNOV MBH
(72)Inventor : OLES MARKUS
SCHLEICH BERNHARD
HOMMES PETER

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(54) STRUCTURIZED SURFACE HAVING PROPERTY FOR INHIBITING CELL PROLIFERATION, METHOD FOR PRODUCING THE SAME AND USE THEREOF

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a structured surface having properties for inhibiting the proliferation of cells and further inhibiting the adhesion of the cells.
SOLUTION: This structured surface is obtained by providing upheaved parts having 50 nm-10 μm average height and 50 nm-10 μm average interval to an unstructured material having ≥20 mN/m surface energy by mechanical embossing, lithographical etching, or molding processing.

LEGAL STATUS

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CLAIMS

[Claim(s)]
[Claim 1] The structuring front face which has the property characterized by said front face having the surface energy of the ridge which has average height of 50nm - 10 micrometers, and average spacing of 50nm - 10 micrometers, and the larger ingredient which is not structured than 20 mN/m to check cell proliferation, in the structuring front face which has the property which checks cell proliferation.
[Claim 2] The structuring front face according to claim 1 where a ridge has average height of 50nm - 4 micrometers.
[Claim 3] The structuring front face according to claim 1 whose average spacing of a ridge is 50nm - 4 micrometers.
[Claim 4] The structuring front face according to claim 1 where a ridge has average height of 50nm - 4 micrometers, and average spacing of 50nm - 4 micrometers.
[Claim 5] The structuring front face given [to claims 1-4] in any 1 term where a ridge has aspect ratios 0.5-20.
[Claim 6] The structuring front face according to claim 5 where a ridge has aspect ratios 1-10.
[Claim 7] The structuring front face according to claim 5 where a ridge has aspect ratios 1-5.
[Claim 8] The structuring front face given [to claims 1-7] in any 1 term currently given on the superstructure in which a ridge has average height of 10 micrometers - 1mm, and average spacing of 10 micrometers - 1mm.
[Claim 9] The structuring front face given [to claims 1-8] in any 1 term which has the subrange by which said front face is not structured additionally.
[Claim 10] The subrange which is not structured is a structuring front face according to claim 9 which has the surface energy of 10 - 20 mN/m.
[Claim 11] The structuring front face given [to claims 1-10] in any 1 term where the ingredient which is not structured contains silicone, the poly dioxane, fibronectin, a collagen, a fibrin, polyurethane, polymethylmethacrylate, polyacrylic acid, a polyvinyl chloride, polyethylene, polypropylene, polyimide, or a polyamide as a homopolymer or a copolymer.
[Claim 12] The structuring front face given [to claims 1-10] in any 1 term where the ingredient which is not structured consists of gold, titanium, quartz glass, lithium niobate, silicon carbide, silicon nitride, a hydroxyl apatite, or silicon.
[Claim 13] The manufacturing method on the front face of structuring which has the property which checks cell proliferation characterized by to emboss mechanically the ridge which has average height of 50nm - 10 micrometers, and average spacing of 50nm - 10 micrometers on the ingredient which has larger surface energy than 20 mN/m, and which is not structured, to etch it by the lithography method, or to give it by fabrication in the approach of manufacturing the structuring front face which has the property which checks cell proliferation.
[Claim 14] The approach according to claim 13 of being simultaneous in a superstructure to the superstructure which has average height of 10 micrometers - 1mm, and average spacing of 10 micrometers - 1mm, embossing a ridge mechanically to it next, etching by the lithography method, or giving by fabrication.
[Claim 15] The method according to claim 13 or 14 of making a ridge have subsequently the

We would like to ask you filing a U.S. patent application based on PCT (entering into U.S. national phase).

Details of this application are as follows.

(1)International application number: PCT/JP 2004/017572

(2)International filing date: 26 , November ,2004

(3)Applicant: TANAKA Masaru, SHIMOMURA Masatsugu, TOYOKAWA Yoshihide (Zeon Medical Inc.)

(4)Due date: 28, May, 2006 (30 months)

The following documents are enclosed.

(1) Copy of the front page of WO 2005/051450 A1

(2)Copy of the International Search Report

(3)English translation of the description, claims, abstract and drawings.

(4)English translation of JP 2001-157574A, JP 2002-347107A, JP 2003-102849A, JP 2004-024616 and JP 2001-149061A, which are cited in the International Search Report.

(5)Copies of Materials Science & Engineering, C: Biomimetic and Supramolecular Systems, 1999,C10(1-2),141-146 and Japanese Journal of Artificial Organs, 2000, Volume 29, Number 1, pp. 257-263, which are cited in the International Search Report.

(6) English translation of JP 2002-335949A, JP 2003-149096A and JP 2001-327609A, which are cited in the Description.

And electric file of the English translations in 3.5 inch FD (MS Word 2003 for Windows XP) are also enclosed. Please confirm them.

We would appreciate your kind cooperation.

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[Claim 15] The method according to claim 13 or 14 of making a ridge have subsequently the

ingredient which has larger surface energy than 20 mN/m.
[Claim 16] The approach given [to claims 13-15] in any 1 term the ingredient which is not structured contains silicone, the poly dioxane, fibronectin, a collagen, a fibrin, polyurethane, polymethylmethacrylate, polyacrylic acid, a polyvinyl chloride, polyethylene, polypropylene, polyimide, or a polyamide as a homopolymer or a copolymer.
[Claim 17] The approach given [to claims 13-15] in any 1 term the ingredient which is not structured consists of gold, titanium, quartz glass, lithium niobate, silicon carbide, silicon nitride, a hydroxyl apatite, or silicon.
[Claim 18] The approach given [to claims 13-17] in any 1 term which covers the ingredient or structuring ingredient which is not structured over plasma treatment.
[Claim 19] The approach given [to claims 13-18] in any 1 term which it makes equipped with the subrange which is mechanical on a structuring front face, or is not structured with lithography.
[Claim 20] Use on the front face of structuring which has the property which checks cell proliferation given [to claims 1-12 as a bioassay for a cell culture container] in any 1 term.
[Claim 21] Use on the front face of structuring which has the property which checks cell proliferation given [to claims 1-12] in any 1 term in cell screening or active substance screening, medicine, plant protection, or toxicology.
[Claim 22] Use on the front face of structuring which has the property which checks cell proliferation given [to claims 1-12 for manufacturing the medical-application implant] in any 1 term.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the microstructure-ized front face on which cell adhesion and cell proliferation are reduced and which has slight surface energy, manufacturing method, and its use.
[0002]
[Description of the Prior Art] The microstructure on a front face is well-known, and is especially used for the front face of self cleaning nature.
[0003] This field has the substantial description of this front face in it being hard to wet wet by water or the aquosity system. or [that the front face which water is made to flow out simply or is crawled as waterdrop is a hydrophilic property very much] -- or it must be hydrophobicity. a hydrophilic front face -- few contact angles with water -- having --; -- this causes quick distribution of the water to a front-face top, and the quick outflow from the front face of the water screen produced in this way succeedingly. use of the hydrophobic ingredient for manufacture of a hydrophobic front face -- well-known --; -- making a front face structure by mm range - nm within the limits has the further development of this front face.
[0004] For example, such a hydrophobic microstructure-ized front face is indicated by H.Seito etc. after Surface Coating International V, 1997, and 168 page. In this publication, it is checking giving the particle which consists of a fluorine polymer on a surface of metal, and reducing strongly the wettability over the water of the front face produced in this way in that case, and reducing an ice-coating inclination remarkably. However, the property which checks the adhesion and growth about a cell culture was not proved in this publication.
[0005] The option to which the wettability of an object is reduced is indicated by by changing surface topography to U.S. Pat. No. 33544022 and an international patent application official announcement/[96th] No. 04123 specification. By these approaches, the artificial upheaval or the artificial impression which has height of 5-100 micrometers and spacing of 5-500 micrometers is given on the ingredient which carried out hydrophobing after a hydrophobic ingredient or structuring. By this kind of front face bringing about quick drop formation, the drop which rolls in that case and falls absorbs a dirt particle, and defecates a front face. For example, the trial in the front face completely soaked in the physiological saline is not indicated. Similarly, the publication about the aspect ratio of a ridge is missing.
[0006] It is well-known that the surface topography within the limits of about 20nm - 50 micrometers (nano topography and micro topography) can affect cell physiology-behavior from the biology which is other technical fields. The range of this magnitude is crossed to the magnitude of a supermolecule and a cell. The topography (macro topography) exceeding 50 micrometers is used as a coarse contact front face of the hard bone implant as the nonwoven fabric and textiles for for example, blood vessel pro TEZEN (Gefaessprotesen), and a porous matrix for the cell culture in within the limits of systems engineering (R.P.Lanza, "Principles of Tissue Engineering", Academic Press, ISBN 1-57059-342-6, Chapter 11).
[0007] Various surface topography is used for intentional control of cell growth. In the case of regular and periodic structure, topography is characterized by the publication of the geometry of

a component and periodicity. Moreover, in the case of the accidental topography used in a cell culture, the statistical publication relevant to distribution (height, a pitch, correlation distance, etc.) of a geometry-parameter is required. The topography of some classes already examined in the cell culture technique is indicated by Biomaterials [/ else / Eisenbart] (1996), 17, 1399-1403, Biomaterials [/ else / Curtis] (1997), 18, 1577-1583, Biomed.Mater.Eng. [/ else / Wen] (1996), 6, and 173-189. The result of this trial is extremely contradictory partially. In spite of having greatly tried hard, it had not succeeded in drawing the general principle for cell growth from surface topography. In spite of having examined already very various geometry structures, periodicity, and a dimension, it had not succeeded in finding out the surface structure which has the property which checks cell adhesion and cell proliferation until now.

[0008] For much use, to use the front face which has the property to reduce cell adhesion and/or cell proliferation was desired.

[0009] Therefore, the colony formation of the bacteria or cell to the front-face top of the pipe line of a big dimension, a container, or packing and diffusion are not desirable. This produces the slime layer which a microorganism population may increase too much, moreover, imitates putrefaction of an article and a consumer's health hazard, and it not only may spoil continuously water quality, drink quality, and food quality, but it may often come.

[0010] From all the important food fields, health should intercept bacteria or a cell. The object for contact with the direct body and the textiles especially for the object for reproductive organs and sick person nursing, or old-man nursing relate to that. a moreover and nursing station -- especially -- within the limits of intensive care and pediatric nursing -- the inside of the inside of a hospital, the room especially for a medical operation, and the isolation station in critical infection -- and a cell and bacteria should be intercepted from the supply front face or the instrument front face in the toilet.

[0011] current, equipment, a supply front face, and textiles -- bacteria or a cell -- receiving -- the need -- responding -- or it should be alike, it has and is processed to some extent as a disinfectant with a wide area and the chemical which acts powerfully and in antimicrobial or its solution, and mixture. or [that such a chemical agent acts nonspecific and it is often the very thing poison] -- or [or / stimulating] -- or the decomposition product in which feels uneasy on health and it deals is formed. Moreover, it ****s and, in the case of the person who did sensitization, incompatibility often appears.

[0012]

[Problem(s) to be Solved by the Invention] Therefore, to make a front face equipped with the property which checks cell proliferation and which reaches and checks/or cell adhesion was desired.

[0013]

[Means for Solving the Problem] In an unexpected thing, it has an aspect ratio with the fixed structure, i.e., the constant ratio of height and average width of face, and the structured front face on which it continues sharply and the colony formation of a cell and spread are reduced was found out.

[0014] Within the limits of this invention, it immortality-izes, and, in addition to this, cells are [no] eukaryotic cells at fibroblast, a primary cell strain, and a row, and bacteria. Bacteria have the adhesion behavior and growth behavior clearly different from a cell.

[0015] The cell to a different ingredient and adhesion of bacteria are very complicated processes, and, as for them, the gestalt is deterministically important for biological functions. Using support protein, in order that a cell may stick to a front face, this makes the orientation movement (migration) of a cell, and diffusion expect. Since a cell has the viability force only in a group, this function is important for the gestalt of a cell. A cell must have been divided in the location, without combining with a front face in a water solution. Although it pastes up on the protein and the front face which were structured simply, bacteria are not for migration and need these protein chiefly rather for protection and optimization of survival conditions. Moreover, fission is performed also in a water solution and it gets. Therefore, growth of the bacteria to a front face is controlled by the device different from the growth to the front face of a cell.

[0016] Therefore, the object of this invention is a structured front face which has the property

which checks cell proliferation, and in that case, there are more front faces than a ridge with an average height [of 50nm - 10 micrometers], and an average spacing of 10nm - 50 micrometers, and 20 mN/m, and they have the surface energy of the ingredient with which 20 - 60 mN/m is not structured especially preferably 20 to 80 mN/m. The front face and ridge of this order are well-known, and are already indicated by the Federal Republic of Germany patent 19803787.No. 2 and the 19914007.3 specification.

[0017] However, these front faces are hydrophobic, as a result strong outstanding water repellence. These have a very high contact angle to water, can be defecated from contamination by rinse by water, or promote the outflow of; or water.

[0018] It is because it, that is, the cell which are not [as for this property] desirable in this ** survive only by the aquosity system, as a result need wetting with a perfect front face.

[0019] Although bacteria and other microorganisms need water for adhesion in a front face, or growth in a front face and there is this [no] in a hydrophobic front face, it is provided on the surface of this invention. According to this invention, the structured front face blocks growth of a cell and;, therefore these check cell proliferation. However, the front face by this invention enables growth limited in the location of the bacteria in the subrange which is not structured, and other microorganisms to the bottom of the ****ing range condition, for example, air humidity and temperature. Inhibition of growth of the cell not an antimicrobital action but on the subrange which is not structured since it is rather based on physical effectiveness is removed for fundamental effectiveness by the structuring range by exsorption (Ausbluten) and/or diffusion of an active substance.

[0020] it can set for physic and biology -- or it is structured -- or use of a **** front face -- for example, others [Eisenbarth / E.] -- "Influence of the surface structure of titaniummaterials on the adhesion of fibroblasts" and Biomaterials 1996, The 17th volume and No. 14, It is examined in the 1399-1403rd page. Here, the number of the pasted-up cells increases independently of the used ingredient which has the increasing granularity, and this is opposed to each other to an operation of this invention. Furthermore, it is shown by R.G.Richard that various granularity does not change adhesion of fibroblast (3 Suppl. the "The effects of surface roughness on fibroblast adhesion in vitro", Injury 1996, the 27th volume, and page 43 of C38 - C).

[0021] Therefore, there is no use of the microstructure-ized front face for reducing cell proliferation and/or cell adhesion in a technical trend.

[0022] This ingredient that is not structured may contain this matter and may consist of this matter only on the front face which it is perfect or should be structured.

[0023] The structuring front face by this invention can be manufactured from very various ingredients, as long as it has larger surface energy than 20 mN/m in the condition that these are not structured. Here, gold, titanium, silicon, carbon, quartz glass, lithium niobate, silicon nitride, silicon carbide, or a hydroxyl apatite is suitable.

[0024] The ingredient which is not structured may consist of this matter only on the front face which may contain this matter, may consist of this matter completely, or should be structured.

[0025] Furthermore, the ingredient which is not structured may consist of this matter only on the front face which may contain the silicone, the poly dioxane, the fibronectin, the collagen, the fibrin, the polyurethane, the polymethylmethacrylate, the polyacrylic acid, the polyvinyl chloride, the polyethylene, the polypropylene, the polyimide, or the polyamide as a homopolymer or a copolymer, respectively, and may consist of this matter completely, or should be structured.

[0026] An ingredient is not poisonous and can be used also in a cell culture technique.

[0027] A surface property decision based on the wettability can be made about measurement of surface energy. With various liquids, this magnitude is smooth, namely, can come to hand about measurement of the contact angle to the ingredient which is not structured (D.K.Owens, R.C.Wendt, J.Appl.Polym.Sci.13, and 1741 (1969)), and is indicated by mN/m (milli newton per meter), for example. According to what was measured by Owens etc., a smooth polytetrafluoroethylene front face has the surface energy of 19.1 mN/m, and the contact angle (advance angle; Fortschreitwinkel) with water is 120 degrees in that case. Generally, a hydrophobic front face has contact - with the water exceeding 90 degrees, or a contact angle

(advance angle). In the case of the surface energy of 29 - 30 mN/m, polypropylene has (it is dependent on the molecular structure, for example, the advance angle over about 105-degree water).
[0028] Measurement of a contact angle or surface energy is performed on a smooth front face according to the purpose, in order to guarantee that it can compare better. Moreover, the chemical composition of the molecular layer of the surface topmost part has determined "hydrophobicity" and "liquid repulsion" which are the property of an ingredient, or "the wetting of a liquid." therefore, an ingredient -- more -- being high -- or -- or a lower contact angle -- or it is more high or lower surface energy can also be attained by the covering approach.
[0029] Therefore, surface hydrophobicity or a hydrophilic property can be defined about surface energy, and it is the scale of surface energy that the contact angle of the ingredient which is not been [an ingredient / it] smooth namely, structured is indicated by mN/m with various liquids, in that case.
[0030] Moreover, the ridge of another dimension can also be used, for example for the specific use field in a cell culture technique. Therefore, in the case of 50nm - 10 micrometers average spacing, there is average height of a ridge preferably at 50nm - 4 micrometers. Alternatively, in the case of 50nm - 4 micrometers average spacing, the average height of a ridge may be 50nm - 10 micrometers. In the case of 50nm - 4 micrometers average spacing, a ridge has height of 50nm - 4 micrometers preferably especially.
[0031] As already touched, the height of a ridge and the ratio of width of face, i.e., an aspect ratio, are very important. a ridge -- 0.5-20 -- desirable -- 1-10 -- you may have the aspect ratio of 1-5 preferably especially. Furthermore, much more chemical composition of the topmost part of an ingredient is important. When using the front face by this invention in a cell culture technique, the limited chemical surface characteristic is needed. A front face must be cell compatibility and must be endotoxin non-**. Moreover, since you may reform after fabricating a front face, the ridge may have larger surface energy than 20 mN/m.
[0032] Moreover, the front face structured by this invention may have the subrange which is not structured and the subrange which has the surface energy of 10 - 20 mN/m especially and which is not structured.
[0033] or [pasting up a cell on the subrange which is not structured] -- or it can be made to increase in this way -- for example, the front face by this invention -- or bioassay cis- (Bioassaysys) or a cell culture trial plate can be made to have by the approach by this invention The subrange which is not structured can be produced with mechanical or lithography.
[0034] or [moreover, / that the subrange which is not structured is covered with the matter which promotes the matter which promotes cell adhesion, for example, a Polly L-amide, and cell growth, for example, fetal calf serum, bovine serum, a goat blood serum, or horse serum] -- or it may be processed.
[0035] Furthermore, the object of this invention is a manufacturing method on the front face of structuring which has the property which checks cell proliferation, in that case, it embosses the ridge which has average height of 50nm - 10 micrometers, and average spacing of 50nm - 10 micrometers on the ingredient which has larger surface energy than 20 mN/m and which is not structured, etches it by the lithography method, or is given by fabrication.
[0036] In order to change a chemical surface characteristic, the approach of producing a radical site (Radikalstelle) can also be further mentioned on a front face. or [having been structured] -- or the ingredient which is not structured may be processed by the special photoinitiator, using the plasma, UV, or a gamma ray. The graft polymerization of the monomer may be additionally carried out after such surface activation, i.e., generation of an uncombined radical. Such an approach especially produces covering which is chemically resistant. As a monomer, acrylate, methacrylate and other vinyl derivatives, for example, methyl methacrylate, ethylene oxide, or a vinyl chloride is applied.
[0037] Surface shaping or structuring is based on embossing/rolling, or is performed to coincidence in the case of macroscopic formation of an object, for example, casting, injection molding, or other fabricating methods. For that, the female mold in which the desired structure ****s is required.

[0038] A female mold can be industrially manufactured for example, with the Riga technique (Ligatechnik) (R.Wechsung in Mikroelektronik, 9, 1995, and the 34th page or subsequent ones). Here, one or more masks are first manufactured with the dimension of a desired ridge by electron-beam lithography. It is used in order to make the photoresist layer according this mask to X ray lithography expose, and thereby, a male is obtained. The space in a photoresist layer is succeedingly filled up according to metaled electrodeposition. In this way, the obtained metal structure is the female mold of the desired structure.
[0039] In another embodiment of this invention, the ridge is arranged on a little coarse superstructure. A ridge can be given on the superstructure which has the above-mentioned dimension and has average height of 10 micrometers - 1mm, and average spacing of 10 micrometers - 1mm. The ridge of a superstructure can be embossed similarly or can be given by the lithography method or fabrication. a ridge and a superstructure -- coincidence -- or -- continuing -- namely, -- first -- a superstructure -- subsequently a ridge can be embossed mechanically, or it can be based on the lithography method, or can give by fabrication.
[0040] In the case of the front face which has a superstructure (for example, the case of the front face which has only a ridge), surface shaping or structuring is performed in a processing phase according to the purpose. The already produced surface subsequent chemical denaturation by which duplex structuring was carried out is possible similarly natural.
[0041] The mechanical approach which introduces structure on the subrange by which it is not structured on the front face which is not structured or the structured front face is the embossing method or the stamp method in the mold or male (needle) manufactured beforehand, for example. The lithography method is the ablation method (ablative Verfahren) not only in for example, the Riga (Liga) technique and X ray lithography but a laser beam.
[0042] The fabricating method is the fluid technique and injection-molding method which are daily use in plastic working.
[0043] The use on the front face of structuring manufactured by the structuring front face or this invention by this invention is another object of this invention.
[0044] active substance screening of new drugs [in / as a cell culture container for a bioassay / in / for the front face by this invention / for example, / cell screening / medicine] -- setting -- or it can be used in plant protection or toxicology. The front face by this invention can also be similarly used for the medical-application implant, for example, a heart valve, or an artificial cardiac pacemaker.
[0045] Although the following examples explain this invention to a detail more, they do not **** the protection range.
[0046]
[Example] The microstructure-ized front face is manufactured by the embossing method. an embossing die (templating mold (Abformwerkzeuge)) -- Liga -- it manufactured by the structuring method based on the basic process of law, i.e., X ray lithography, electroplating, and templating (Roentgen-LIthographie, Galvanik und Abformung). The structure did not arise in the base material according to an etching process, but rather, concerning a mold, this approach is an injection-molding method, did not require costs, but it is the point which carries out templating and differed from microscopic dynamics (Mikromechanik). After resist exposure (radiosensitivity polymer) and development of lithography, the lacquer structure (Lackstructur) produced in this way is used as a mold for an electroplating process, and a metal is deposited in the crevice exposed at the process. Then, the lacquer structure is removed and the metal structure to which the remainder remained is used for a templating mold (G.Gerlach, W.Doetzel"Grundlagen der Mikrosystemtechnik"Carl Hanser Verlag Muenchen, 1997, and the 60th page or subsequent ones).
[0047] In this way, the manufactured structure has only the fine structure which has average height of 4 micrometers, and average spacing of 3 micrometers in the case of the analyte 1. The structure of analyte 2-4 has the superstructure which has average height of 20 micrometers, and average spacing of 32 micrometers additionally. The fine structure ****s in the analyte 1.
[0048] Drawing 4 shows the REM image (analyte 4) which has a superstructure and the fine structure. Based on the depth of field to which the REM image was limited, the fine structure is

checked on "a crowning (Spitzen)" of a superstructure, and it deals in it.
[0049] In this way, the disk which has the diameter of 9mm is cut off and it moves from the microstructure-ized polycarbonate-front face to the cell culture pallet (Nunk, catalog number 167008) of 96 wells. The structured field is shown upwards in that case. Under the bench (Arbeitsbank) which sterilized, passage 2 inoculates human skin fibroblast into this ingredient (3x104 cells / ml, 100microl / well). Using various assays, the growth behavior of a fibroblast-cell is evaluated and the thing of it can be carried out. The ingredient with which similarly the front face which inoculated human skin fibroblast is not structured is used as contrast. The cell fractions which have not been pasted up were collected about various days. It is shown that cells fewer 20 to 40% than a pars basilaris ossis occipitalis pasted the microstructure-ized front face. Drawing 1 shows the number of the survival cells pasted up on the front face. The proliferation rate and the vital force of all culture were measured using MTT-assay. This assay is based on returning in the reaction to the formazan which the cell absorbed tetrazolium salt and was dependent on the mitochondrion. After the dissolution of a cell, this color can separate and can carry out a quantum with a photometry. Since only the incorporation by the mitochondrion can perform MTT reduction, measured value can do the reasoned thing to the vital force and cell proliferation. Evaluation of MTT measurement of the pasted-up cell showed that the cell growth on the microstructure-ized front face decreased clearly as compared with the front face of contrast about various days. As compared with the front face where cell proliferation is not structured, there was also little 20% partially (refer to drawing 2 , MTT assay.). Relative Ko maximum.
[0050] The rate of the pasted-up cell of having survived and died was measured using the live / dead (live/dead)-fluorescein assay. It is not the case of the surface-structure-ized sample, either, and, also in the case of contrast, the remarkable difference was shown in the ratio of a survival cell and a death cell (drawing 3), namely, cell adhesion or cell proliferation was controlled effectively, without killing a cell. Therefore, a toxic operation of the structured front face can be eliminated.
[0051] The introductory notes d1, d3, d5, and d8 to drawing 1 -3 express after [1, 3, and 5] inoculation or the 8th day.
[0052] "Contrast" expresses the specimen which consists of the same ingredient, without carrying out surface structure-ization by this invention.
[0053] Analyte 1, 2, 3, and 4 is following parameter: [0054].
[Table 1]

	上部構造 [μm]			微細構造 [μm]		
	A	B	C	A	B	C
分析物1	-	-	-	3	4	1
分析物2	24	15	10	3	4	1
分析物3	15	15	7	3	4	1
分析物4	32	20	12	3	4	1

[0055] A structuring front face different, respectively which **** is expressed (about A, B, and C, it is referring to drawing 5).
[0056] Drawing 1 shows adhesion assay and has indicated the cell which per one analyte pasted up to the y-axis in that case. The ****ing absolute number becomes clear to the basis of a bar graph, respectively. Respectively, in the value of d1, and the rod of middle, the value of d2 and the right-hand side rod express [the left rod] the value of d3.
[0057] Drawing 2 shows MTT assay and has indicated the percentage of a survival cell to the y-axis in relation to contrast assay in that case. The ****ing number of percents becomes clear to the basis of a bar graph, respectively. The rod has expressed the value of d1, d3, d5, and d8 from the left to the right, respectively.
[0058] Drawing 3 shows the live / dead-assay, and has indicated the percentage of a survival cell to the y-axis in that case. This ****ing number of percents becomes clear to the basis of a bar graph, respectively. The rod has expressed the value of d1, d3, d5, and d8 from the left to the right, respectively.

[Translation done.]